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(54) Title: 97 HUMAN SECRETED PROTEINS

(57) Abstract

The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel human secreted proteins.

260 265

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Gln Ser Ser Pro Pro Pro Gln Pro His Pro Cys His Thr Cys Arg Gly
35 40 45

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Lys Asp Ser Glu Thr Arg Leu Val Glu Val Leu Glu Gly Val Cys Ser
85 90 95

Lys Ser Asp Phe Glu Cys His Arg Leu Leu Glu Leu Ser Glu Glu Leu
100 105 110

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115 120 125

Gln Trp Leu Cys Ser Asp Ser Leu Lys Leu Cys Cys Pro Ala Gly Thr
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Phe Gly Pro Ser Cys Leu Pro Cys Pro Gly Gly Thr Glu Arg Pro Cys
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Gly Leu Gly Tyr Phe Glu Ala Glu Arg Asn Ala Ser His Leu Val Cys
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Gln Phe Cys Val Asn Thr Glu Gly Ser Tyr Glu Cys Arg Asp Cys Ala
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Cys Ser Pro Gly Tyr Gln Gln Val Gly Ser Lys Cys Leu Asp Val Asp

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    290                 295                 300

Glu Cys Glu Thr Glu Val Cys Pro Gly Glu Asn Lys Gln Cys Glu Asn
305                 310                 315                 320

Thr Glu Gly Gly Tyr Arg Cys Ile Cys Ala Glu Gly Tyr Lys Gln Met
                325                 330                 335

Glu Gly Ile Cys Val Lys Glu Gln Ile Pro Gly Ala Phe Pro Ile Leu
            340                 345                 350

Thr Asp Leu Thr Pro Glu Thr Thr Arg Arg Trp Lys Leu Gly Ser His
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Pro His Ser Thr Tyr Val Lys Met Lys Met Gln Arg Asp Glu Ala Thr
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Phe Pro Gly Leu Tyr Gly Lys Gln Val Ala Lys Leu Gly Ser Gln Ser
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Arg Gln Ser Asp Arg Gly Thr Arg Leu Ile His Val Ile Asn Ala Leu
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<221> SITE
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<223> Xaa equals any of the naturally occurring L-amino acids

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Phe Leu Gly Leu Ser Ala Leu Ala Pro Pro Ser Arg Ala Gln Leu Gln
             20                  25                  30

Leu His Leu Pro Ala Asn Arg Leu Gln Ala Val Glu Gly Gly Glu Val
         35                  40                  45

Val Leu Pro Ala Trp Tyr Xaa Leu His Gly Glu Val Ser Ser Ser Gln
     50                  55                  60

Pro Trp Glu Val Pro Phe Val Met Trp Phe Phe Lys Gln Lys Glu Lys
 65                  70                  75                  80

Glu Asp Gln Val Leu Ser Tyr Ile Asn Gly Val Thr Thr Ser Lys Pro
                 85                  90                  95
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invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 102-120, 121-140, 141-160, or 161 to the end of the coding region. Moreover, polypeptide fragments can be about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes.

Preferred polypeptide fragments include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotide fragments encoding these polypeptide fragments are also preferred.

Also preferred are polypeptide and polynucleotide fragments characterized by structural or functional domains, such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Polypeptide fragments of SEQ ID NO:Y falling within conserved domains are specifically contemplated by the present invention. Moreover, polynucleotide fragments encoding these domains are also contemplated.

Other preferred fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Epitopes & Antibodies

In the present invention, "epitopes" refer to polypeptide fragments having antigenic or immunogenic activity in an animal, especially in a human. A preferred embodiment of the present invention relates to a polypeptide fragment comprising an epitope, as well as the polynucleotide encoding this fragment. A region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." In contrast, an "immunogenic epitope" is defined as a part of a protein that elicits an antibody response. (See, for instance, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998- 4002 (1983).)

Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, R. A., Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985) further described in U.S. Patent No. 4,631,211.)

In the present invention, antigenic epitopes preferably contain a sequence of at least seven, more preferably at least nine, and most preferably between about 15 to about 30 amino acids. Antigenic epitopes are useful to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe, J. G. et al., Science 219:660-666 (1983).)

Similarly, immunogenic epitopes can be used to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., supra; Wilson et al., supra; Chow, M. et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle, F. J. et al., J. Gen. Virol. 66:2347-2354 (1985).) A preferred immunogenic epitope includes the secreted protein. The immunogenic epitopes may be presented together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse) or, if it is long enough (at least about 25 amino acids), without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting.)

As used herein, the term "antibody" (Ab) or "monoclonal antibody" (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')2 fragments) which are capable of specifically binding to protein. Fab and F(ab')2 fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody. (Wahl et al., J. Nucl. Med. 24:316-325 (1983).) Thus, these

fragments are preferred, as well as the products of a FAB or other immunoglobulin expression library. Moreover, antibodies of the present invention include chimeric, single chain, and humanized antibodies.

Fusion Proteins

Any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target cellular locations based on trafficking signals, the polypeptides of the present invention can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

Moreover, polypeptides of the present invention, including fragments, and specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP A 394,827; Traunecker et al., Nature 331:84-86 (1988).) Fusion proteins having disulfide-linked dimeric structures (due to the